Particle Analysis in Wastewater Effluent

by

Muhammad Haziq Bin Mohamad Dzafer
13230

Dissertation submitted in partial fulfillment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

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Universiti Teknologi PETRONAS

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CERTFICATION OF APPROVAL

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Approved by,

_____

(Dr. Taslima Khanam)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
MAY 2014

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, tha the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

(MUHAMMAD HAZIQ BIN MOHAMAD DZAFER)

ABSTRACT

This research paper is to study effect of image analysis in POME wastewater plant. This topic is very relevant to the Malaysia current industry as there is still no certain research done relating both POME sample with imaging technique. The rate at which other environmental problems are mounting is also alarming. The activated sludge is the major component in treating wastewater and where microorganism's characteristics are observed. Poor wastewater treatment and poor floc settling are examples of less efficient water treating process. The world water quality is worsening and our river and sea are being contaminated with this chemical wastes.

In order to fulfill the objective of this research in which to study the size of aggregates by using the POME sample, number of experiments and microscopic analysis need to be conducted. The analysis that are need to undergone are the effect of serial dilution, image processing, and also percentages of aggregate size. The main purpose of this analysis is to determine the best properties out from the POME wastewater effluent system to be applied in their activated sludge. The length of this research is up to nine months starting from January 2014 until September 2014.

ACKNOWLEDGEMENT

First and foremost, thanks to God for His blessing and mercy in completing this one year project. It would befit to extend my outstretched gratitude to Dr. Taslima Khanam, Chemical Engineering Department, Universiti Teknologi PETRONAS. It is a privilege to be under her supervision. Even with his tight schedules as lecturer and high commitment to Universiti Teknlogi PETRONAS, there was no moment where she fails to provide support and guidance. Her advices and moral support gave a sense of strength and confidence in conducting the final year project.

Many thank to our Final Year Project Coordinators, for their unlimited contributions success in providing the students with guidelines and seminars to enlighten hopes of confidence. Not forget to thank all lab executive and technicians as their willingness to provide the facilities and entertain our demand during conducting the project.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

A biological wastewater treatment plant (WWTP) can be considered an artificial ecosystem consisting of abiotic and biotic components interacting. The abiotic components are represented by the plant and the sewage, whereas the biotic components comprise the decomposers (bacteria and fungi) that take energy for their growth from the dissolved organic matter and the oxygen in incoming wastewater and by protozoa and metazoa microfauna grazing on the decomposers [8]. Usually, the identification and quantification of each protozoa and metazoa species is achieved by microscopic inspection and manual counting, requiring both time and high technical expertise. However, the technological advances and the decrease of computation costs gave the opportunity for new techniques such as image analysis to be used in routine classification and quantification of microorganisms in an automated and non subjective manner overcoming some of the drawbacks of manual techniques. In image analysis, the inherent accuracy and precision of microscopy techniques and the speed of the hardware computation are combined thus reducing the human error factor [9]. Image analysis nowadays a very important tool with a large field of applications due to its ability to remove the subjectiveness of human analysis, the possibility to extract quantitative data and avoid tedious and highly time-consuming tasks to human researchers [1] and quantitative image analysis techniques have been increasingly used throughout the years for the assessment of aggregates and filamentous bacteria properties [2]. The purpose of this experiment is to study the effect of operating parameters in the characterization of aggregates structure and filamentous bacteria determination in wastewater using image analysis. An automated image analysis seems to be an appropriate method to characterise quantitatively both aggregates and filamentous bacteria. From the survey of available resources in the university and time consumption for the project, the imaging technique is the best experiment to be tested. By proper planning of the project, the imaging analysis process will improve the data gathering technique for activated sludge system and by the end of the project, results from the experiment will reveal a better view of filamentous bacteria and aggregates character monitoring. Vast development of science and technology nowadays is a perfect stages for the applied science of microscopic analysis and it just may open a whole new level of engineering exploration.

1.2 PROBLEM STATEMENT

Image analysis, in a wide sense, can be referred to both the strictly image analysis processes as well as to the overall processes of image capture, processing and analysis. This technique allows images improvement as well as automatic recognition and identification of patterns, such as arrangements or groups of elements that follow certain characteristics, resulting in a reduction of time and work. Indeed, image analysis has already proved to be a potentially alternative tool to overcome the drawbacks associated to the micro-organisms visual identification and quantification, and in this case the micro-organisms are reffering to the aggregates and filamentous bacteria characterization in wastewater plant.

However, rather than tiring, imprecise and time-consuming classical methods to characterize aggregates and evaluate filamentous bacteria contents by manual counting under a microscope, microscopic images analysis can be performed in a 1–2 hour period for a total of 100–200 images, whilst the images treatment, data determination and analysis could be recorded in 2–3 hour.

The decrease of computation costs gave the opportunity for new techniques such as image analysis to be used in routine classification and quantification of microorganisms in an automated and non subjective manner overcoming some of the drawbacks of manual techniques. In image analysis, the inherent accuracy and precision of microscopy techniques and the speed of the hardware computation are combined thus reducing the human error factor.

From these results it may be inferred that the current methodology can be used to predict at some extent, critical wastewater treatment plant conditions in a feasible time period (within few hours) and without the need of specialized personnel.

1.3 OBJECTIVE

The aim of the project is to study the efficiency of particle image analysis in the characterization of aggregates structure and filamentous bacteria in palm oil mill effluent (POME) wastewater sample.

1.4 SCOPE OF STUDY

The experiment will use 30 watt halogen illumination which provides enhanced image quality and brightness for the observation of specimens, followed by photo microscopy as a medium to obtain the designated images of aggregates structure and filamentous bacteria which will be going through an automated image analysis for particle image analysis. ImageJ software is used to enhance and normalize the image before the sum of the pixels of the projected surface of aggregates within an image is obtain. The experiment will operate under the parameter of exploring quantitative measurement of aggregates and filamentious bacteria in palm oil mill effluent wastewater plant efficiency.

1.5 RELEVANCY OF PROJECT

This project is important as it deals with current issue in Malaysia palm oil industry which is the wastewater treatment. Microsscopic analysis is believed to be one of the effective ways to replace the conventional microorganisms identification present in the activated sludge of wastewater treatment plants. Hence, this project is relevant as image analysis for POME wastewater has not been widely addressed yet.

1.6 FEASIBILITY OF PROJECT

This project is feasible as it deals with narrowed scope of experiment whereby only 2 parameters are tested. It is within capability to be executed with helps and guidance from the supervisor and the coordinator. With the acquirement of equipment and materials needed, this project is managed to be completed within the time allocated.

CHAPTER 2

LITERATURE REVIEW

2.1 Abstract

Microorganisms are an essential part of the wastewater treatment process. They are added to the aeration chamber and allowed to digest organic matter in the water [3]. Then they are removed in the clarifier. Some of the microorganisms from the clarifier are added back to the aeration basin to start the process over again. The use of microorganisms to remove dissolved organic materials from the water is a form of secondary treatment of the wastewater.

2.1.1 Activated Sludge

The sludge from the clarifier is not simply piped back into the aeration basin. Instead, the sludge must be aged and stressed, or **activated**. Each day's sludge (also known as floc) is pumped out of the clarifier and into a holding basin. There, it is added to the top of the previous day's sludge, as shown below. The sludge is allowed to age for ten days before it is pumped as seed to the aeration chamber.

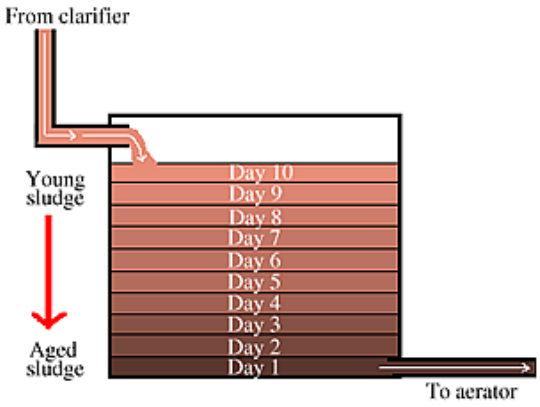


Figure 1 : 10 days aged of activated sludge

The 10 days which sludge is aged is very important. Aging stresses the sludge. Without any food, the microorganisms in the sludge become very hungry. As a result, when they are added to the aeration chamber, they are ready to feed again and are able to reduce the BOD in the water in a short period of time. Stressing the microorganisms also prompts them to multiply rapidly. This results in even more microorganisms to quickly eat up the organic matter in the wastewater. Packaged plants and extended aeration plants are both able to remove BOD in about two hours or less because of the aged sludge used. The aged sludge provides optimum food removal and results in a good quality effluent.

2.1.2 Sludge Composition

The type of microorganisms are found in sludge by looking at the sludge under a microscope. For example, if the organisms found in sludge of various ages under a microscope at 400 power, it might look like this:

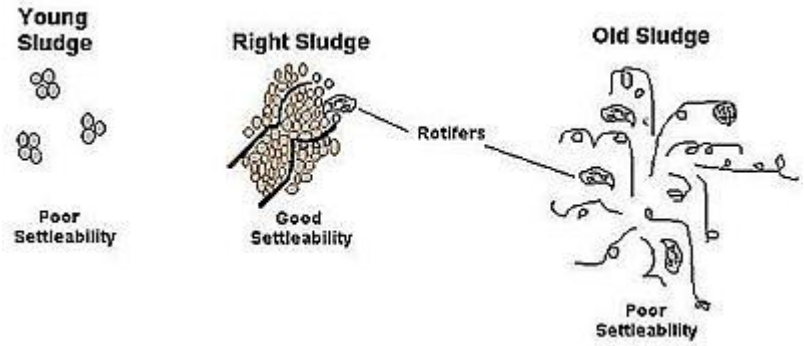


Figure 2 : Rotifiers (aerobic microorganisms) in sludge

Note the **rotifers**, large aerobic microorganisms found in old sludge. Sludge of the right age has a few rotifers in it, but young sludge has none and old sludge has too many. Conditions other than age will also influence the composition of the sludge. As shown in the picture below, low pH, high grease, low nitrogen, and low phosphorus conditions will result in foaming sludge with few rotifers.

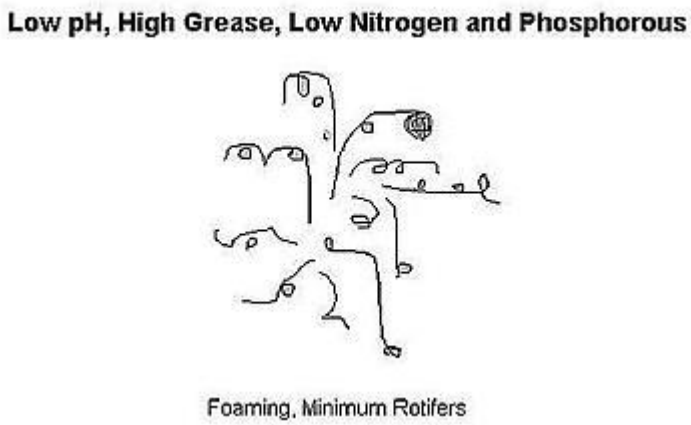


Figure 3 : Example of few rotifers sludge

Anaerobic organisms are also present in sludge. The picture below shows anaerobic organisms which will produce good settleability of sludge.

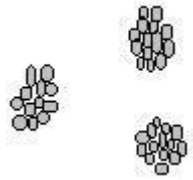


Figure 4 : Example of good settleability sludge

All of the cells in the picture above are of the same cell type. Sticky enzymes on the cells cause them to clump together, which will make them settle out well in the clarifier.

2.1.3 Filamentous Bacteria

Filamentous bacteria serve as the backbone of floc formation. Sludge settles most efficiently when it contains a moderate number of filaments which provide structure for the floc and aid in the stripping of the water column. The floc cannot form properly if there are too few filaments, and the floc cannot settle properly if there are too many [4]. The filamentous bacteria are analyzed in two ways: their effect on floc structure and their abundance. In small amounts, they are quite good to a biomass, but in large amounts they can cause many problems.

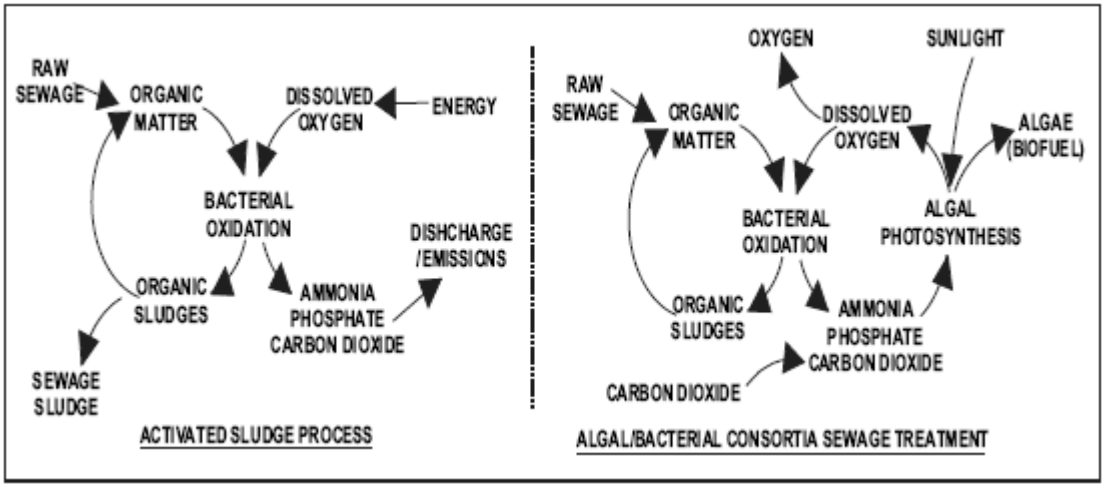


Figure 5 : Basic biological processes in wastewater treatment, illustrating the benefits of algal/bacteria

There are a number of types of filamentous bacteria which proliferate in the activated sludge process. Filamentous organisms perform several different roles in the process, some of which are beneficial and some are detrimental. When filamentous organisms are in low concentrations they serve to strengthen the floc particles. This effect reduces the amount of shearing in the mechanical action of the aeration tank and allows the floc particles to increase in size. Larger floc particles are more readily settled in a clarifier. Larger flow particles

settling in the clarifier also tend to accumulate smaller particulates (surface adsorption) as they settle, producing an even higher quality effluent. Conversely, if the filamentous organisms reach too high a concentration, they can extend dramatically from the floc particles and tie one floc particle to another (interfloc bridging) or even form a filamentous mat of extra large size. Due to the increased surface area without a corresponding increase in mass, the activated sludge will not settle well. This results in less solids separation and may cause a washout of solid material from the system. In addition, air bubbles can become trapped in the mat and cause it to float, resulting in a floating scum mat. Due to the high surface area of the filamentous bacteria, once they reach an excess concentration, they can absorb a higher percentage of the organic material and inhibit the growth of more desirable organisms.

Filamentous bacteria have many positive aspects, such as:

- They are very good BOD removers.
- They add a backbone or rigid support network to the floc structure.
- Helps the floc structure to filter ut fine particulate matter that will improve clarifier efficiency.
- They help the floc to settle if in small amounts.
- They reduce the amount of "pin" floc.

They also have severyal negative aspects, such as:

- They can interfere with separation and compaction of activated sludge and cause bulking when predominant.
- They can affect the sludge volume index (SVI) (Sludge Volume Index).
- They can cause poor settling if dominant.
- They can fill up a clarifier and make it hard to settle, causing TSS (Total Suspended Solids) carryover.
- They can increase polymer consumption.
- They can increase solids production and cause solids handling costs to increase significantly.

2.1.4 Total Suspended Solids (TSS)

Total suspended solids (TSS) include all particles suspended in water which will not pass through a filter. Suspended solids are present in sanitary wastewater and many types of industrial wastewater. There are also nonpoint sources of suspended solids, such as soil erosion from agricultural and construction sites. As levels of TSS increase, a water body begins to lose its ability to support a diversity of aquatic life. Suspended solids absorb heat from sunlight, which increases water temperature and subsequently decreases levels of dissolved oxygen (warmer water holds less oxygen than cooler water) [2]. Some cold water species, such as trout and stoneflies, are especially sensitive to changes in dissolved oxygen. Photosynthesis also decreases, since less light penetrates the water. As less oxygen is produced by plants and algae, there is a further drop in dissolved oxygen levels.

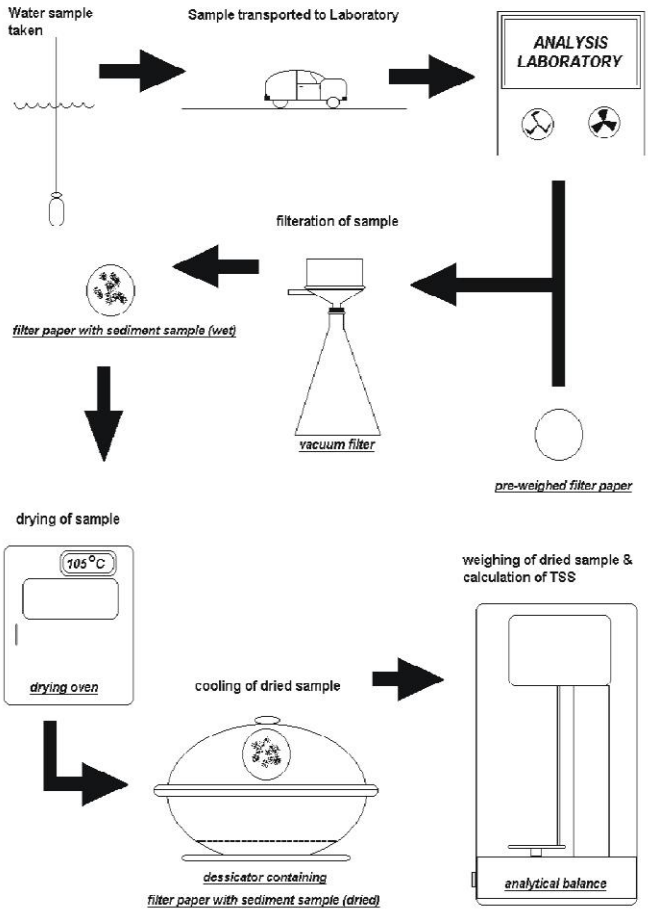


Figure 6: Analysis of Total Suspended Solids

2.1.5 Sludge Volume Index (SVI)

Sludge Volume Index (SVI) is an indication of the sludge settleability in the final clarifier. It is a useful test that indicates changes in the sludge settling characteristics and quality. By definition, the SVI is the volume of settled sludge in millliliters occupied by 1 gram of dry sludge solids after 30 minutes of settling in a 1000 ml graduated cylinder or a settleometer [5].

A liter of mix liquor sample is collected at or near the outlet of the aeration tank, settled for 30 minutes in a 1 liter graduated cylinder, and the volume occupied by the sludge is reported in milliliters.

The SVI is computed by dividing the result of the settling test in ml/liter by the MLSS concentration in mg/L in the aeration tank times 1000.

$$SVI = \frac{\text{Settled Sludge Volume in ml/L after 30 min.} \times 1{,}000}{\text{MLSS, mg/L}} = \frac{\text{ml}}{\text{gram}}$$

or

$$SVI = \frac{\text{\% Settleable at 30 min. x 10,000}}{\text{MLSS}} = \frac{\text{mL}}{\text{gram}}$$

The common range for an SVI at a conventional activated sludge plant should be between 50 and 150. Optimum SVI must be determined for each experimentally.

Sludge Density Index is used like the SVI to determine sludge settling characteristics and return sludge pumping rates. SDI is computed by:

$$SDI = \frac{\text{MLSS \% x 100}}{\text{\% Volume occupied by MLSS after 30 min. settling}}$$

The common operational range for SDI is 1.0 - 2.5. The SVI and SDI indexes relate the weight of sludge to the volume that the sludge occupies and attempts to show how well the activated sludge separates from the mix liquor. Sludges with a low SVI (high SDI) have good settling and compaction characteristics.

2.1.6 Quantitative Image Analysis

Image analysis is the extraction of meaningful information from images; mainly from digital images by means of digital image processing techniques [6]. Main steps of image analysis are image capturing, image storage (compression), correcting imaging defects (e.g. non-uniform illumination, electronic-noise, and glare effect), image enhancement, segmentation of objects in the image and image measurements. The brightness of an image represented by its grey values can be analyzed for every single pixel or for a group of pixels. The most frequently used pixel-based image descriptors are optical density, and integrated optical density.

2.1.7 Image Segmentation

The goal of segmentation is to simplify and/or change the representation of an image into something that is more meaningful and easier to analyze. More precisely, image segmentation is the process of assigning a label to every pixel in an image such that pixels with the same label share certain visual characteristics. Throughout the experiments, image segmentation would be applied in the Image J software during the pre-processing step of filaments segmentation.

2.1.8 Thresholding

The simplest method of image segmentation is called the thresholding method. This method is based on a clip-level (or a threshold value) to turn a gray-scale image into a binary image. The key of this method is to select the threshold value (or values when multiple-levels are selected). This step is purposely for the debris elimination, resulting different intensity value for each of the different filaments found in the binary image [7].

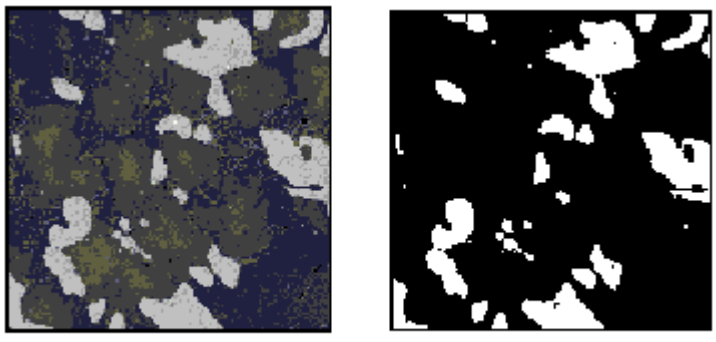


Figure 7: An example of image with thresholding technique

CHAPTER 3

METHODOLOGY

3.1.1 Project Flow Chart

Literature Review

- For FYP II, further understanding on the identification and comparison between image analysis technique and its methodology.
- More review on literature is done to understand the automatic particle count in speeding up the image analysis process.

Experiment

- Experiment is designed to test dilution technique in treating POME wastewater sample.
- Apparatus and materials required for the experiment is prepared and the experiment is currently ongoing.

Data Collection

- From the experiment conducted, the samples are withdrawn.
- The samples properties are observed using thresholding, binary and the analyze particles in ImageJ software.

Conclusion

- The experiment will be concluded based on the results at the end of the project.
- Report for the project will be prepared.

Figure 8: Project flow chart

3.1.2 Key Milestones and Project Gantt chart

No	Detail Work	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Dilution Experiment														
2	Processing the image collected														
3	Measure and tabulate data														
4	Submission of Progress Report														
5	Pre-SEDEX														
6	Submission of Final Draft Report														
7	Submission of Dissertation (soft bound)														
8	Submission of technical paper														
9	Viva														
10	Submission of Project Dissertation														

Figure 9: Project Gantt chart

3.2 EXPERIMENT METHODOLOGY

3.2.1 Experiment Setup

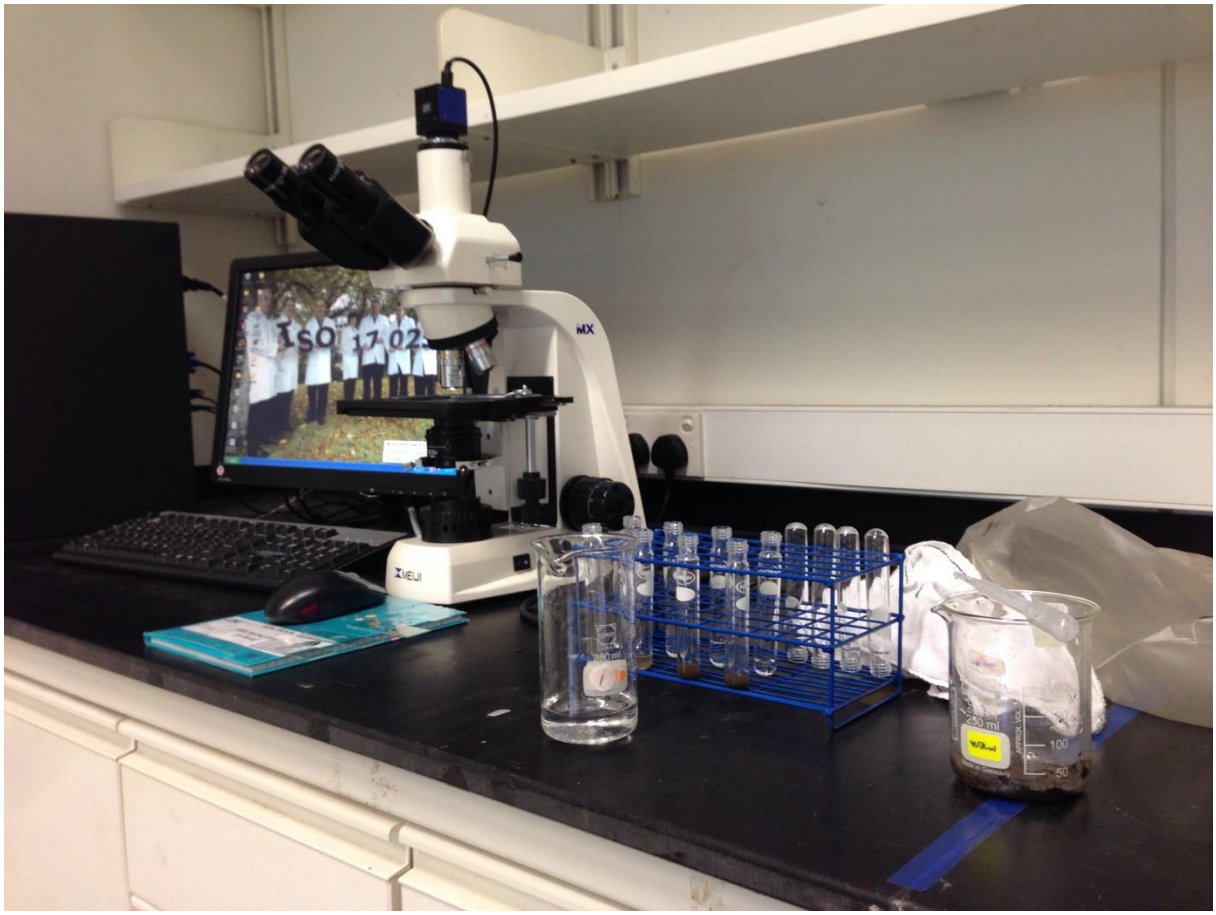


Figure 10: Microscopic working Area in Block 5

3.2.2 Diluting the sample:

POME wastewater sample is prepared by diluting the sample in distilled water. The sample is diluted for every run of experiment in order to get clear and better image acquisition. A dilution is a common laboratory technique used to obtain the desired concentration. A dilution will always reduce the concentration of the sample. Dilutions are ratios and are generally expressed in terms of whole numbers and are reduced to the lowest common denominator. The dilution ratio can be defined as the volume of sample per total volume. The total volume is equal to the volume of the sample plus the volume of the buffer used to make the dilution. Examples of a 1/2 and 1/10 dilutions are shown in **Figure 11**.

Example of ways to make a ½ dilution

$$\frac{1}{2} = \frac{1}{1+1}$$

$$\frac{10}{20} = \frac{10}{10+10}$$

$$\frac{100}{200} = \frac{100}{100+100}$$

$$\frac{0.1}{0.2} = \frac{0.1}{0.1+0.1}$$

$$\frac{0.15}{0.3} = \frac{0.15}{0.15+0.15}$$

Figure 11: Calculating dilution

The starting concentration of the sample was 10 particles/mL with a 1/2, and 1/10 dillutions the final concentrations are 5 particles/mL and 1 particle/mL repectively

Since dilutions are ratios, there are infinite number of ways to make the same dilution. When the numerator and denominator of a dilution are multiplied by the same number have the same dilution with a different total volume. The way in which make the dilution depends on the volume of sample needed for the experiment. It has been known that if using a larger volume we obtain a more accurate dilution. So for better results, use 1:1000 dilution. And that is by adding 1ml of sample to 999 ml of diluent. But practically its impossible to use 999 ml of diluent. So serial dilution is much better option to be applied throughout the experiment.

Serial Dilution is a dilution made of a series of smaller dilution, and the total dilution is the product of each dilution in the series. When large dilution are required, the dilution cannot be made in one step. Since dilutions are ratios we can treat them mathamatically by making serial dilutions. **Figure 12** represents different way of making serial dilutions.

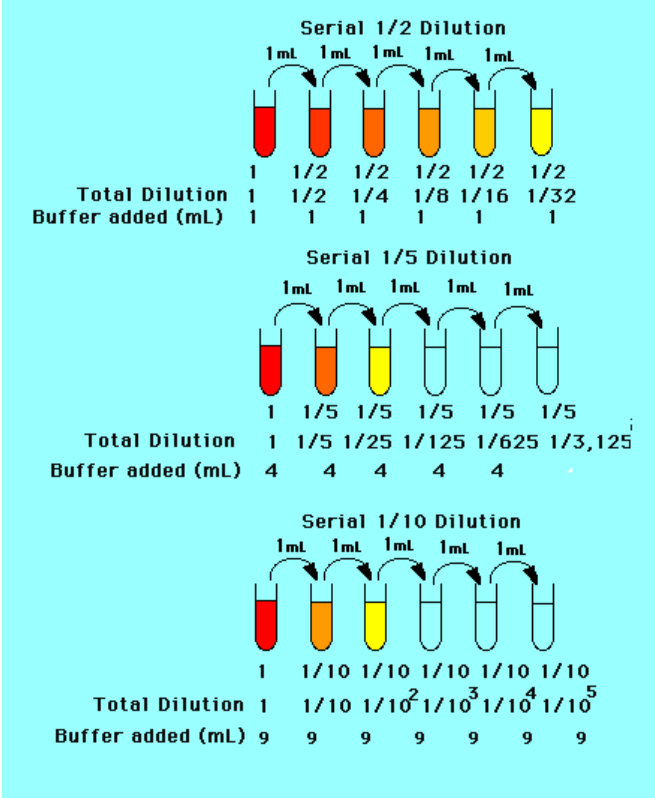


Figure 12: Serial dilution

3.2.3 Image acquisition

For image acquisition, a volume of 500 µL from each sample was distributed on a slide and covered with a 20mm×20mm cover slip for visualization and image acquisition. The sample distribution was performed by means of washing micropipette with a distilled water to prevent contamintaion and also allowing the passage larger aggregates. During the experiment, three different slides (replicates) were screened in order to minimize sampling errors, and images were acquired for 22 different positions in the upper, middle and lower parts of the slide. Therefore, a total around 200 images per sample (22×3 positions×3 replicates) were acquired in bright field microscopy to obtain representative information ,of the sludge. All the images were acquired in a MEIJI microscope, model : MX4300L, with 4x, 10x, and 40x magnification, coupled to a 800k digital camera. The image acquisition was performed in 1024×768 pixels and 8-bit format through the ViS ver .2.90 software.

3.2.4 Image processing

The image processing and analysis program for aggregated and filamentous bacteria characterization was developed in ImageJ Version 1.47 (“Ferreira, T. & Rasband, W., The

ImageJ User Guide — Version 1.47, Apr 2010). Primarily, the image processing step established the binary images from the aggregated biomass and freely dispersed filamentous bacteria and thereafter, morphological parameters were determined. In total, and during the current work, from the overall 142 samples dataset of the POME WWTPs, was acquired and individually characterized. **Fig. 13** shows a schematic representation of the main steps of the program, comprising the image pre-treatment, segmentation, and debris elimination whereas the image analysis program was oriented to the aggregated and filamentous bacteria characterization and contents determination.

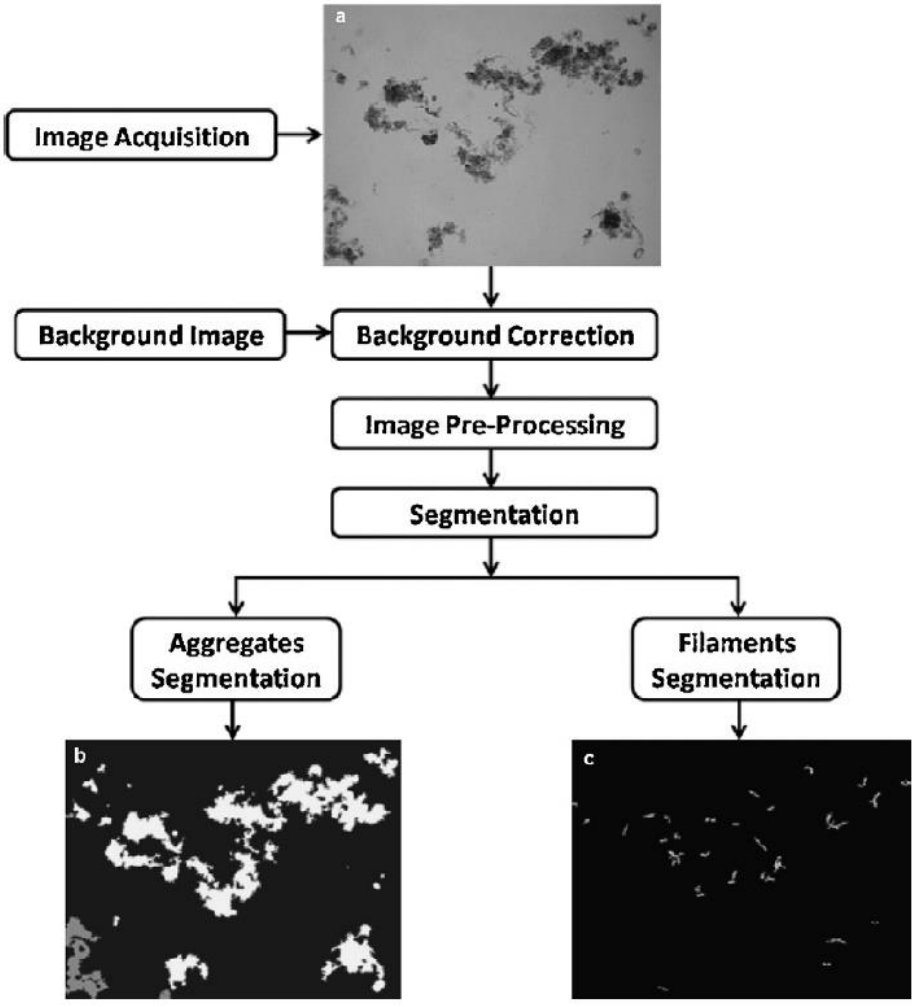


Figure 13: a schematic representation of the main steps of the program

For clarity purposes, according to the physical and morphological meaning of each parameter, in five main descriptor groups: free filamentous bacteria contents; free filamentous bacteria characterization; aggregates content; aggregates size and aggregates morphology. Furthermore, for the aggregates content, aggregates size, and aggregates morphology, a more detailed analysis was performed, including data of all aggregates, labeled as overall (ovr),

intermediate (int) and large aggregates (lrg). For the determination of these values, the average (mean value) of, respectively, all (ovr), intermediate (int) and large (lrg) individual aggregate parameters, was determined. Due to the poor pixel representation of the small aggregates within.

3.2.5 Experiment Procedure

The image grabing is conducted with the microscope for about 20 minutes. Taken the previous research work as guidance (A.L. Amaral, E.C. Ferreira, 2005), the procedure for image analysis with POME wastewater sample are:

1. 20ml solution of wastewater with 50ml of distilled water was collected a beaker.

2. The distilled water then are prepared in 4 different test tube measured by micropipette. The volume was fixed at 1ml. Another beaker filled with distilled water then are used for cleaning the micropipette.

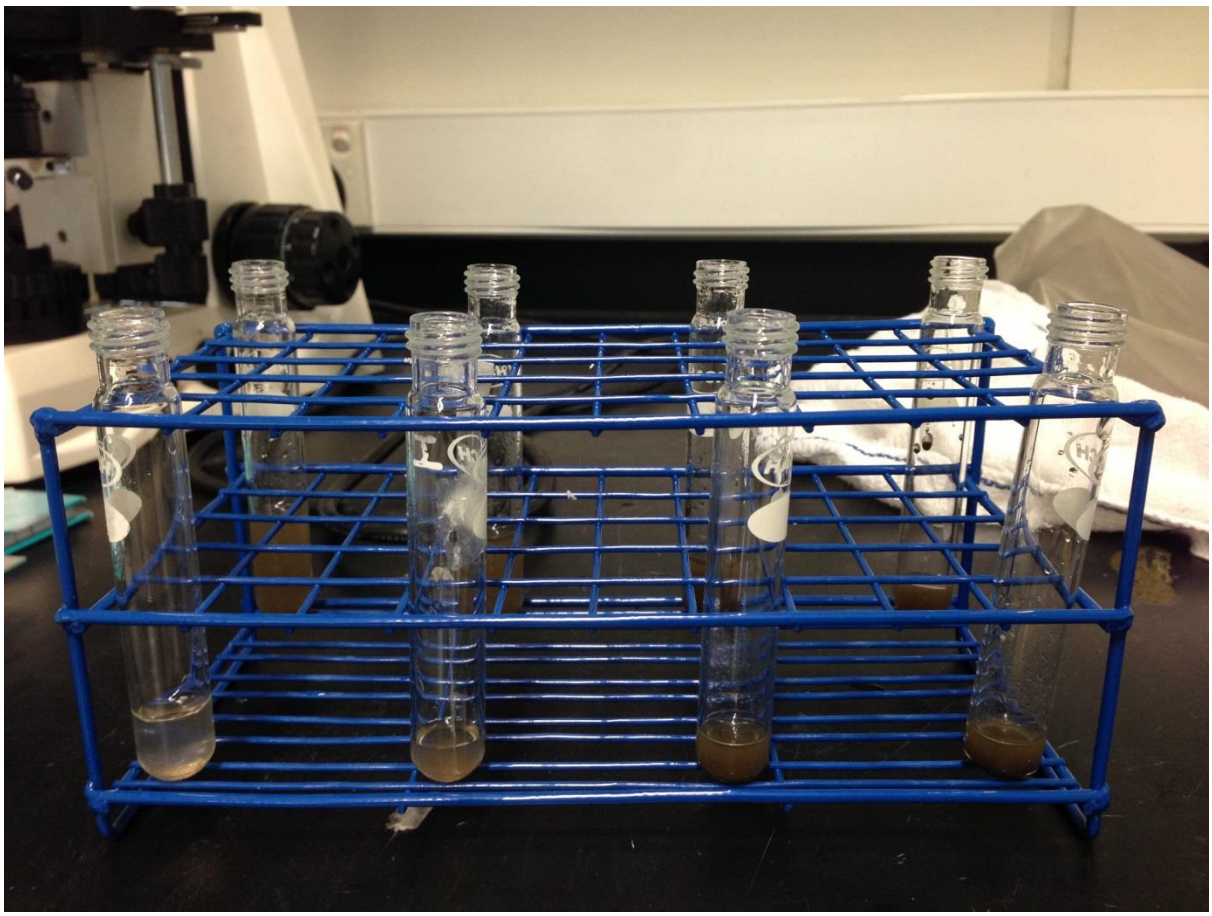


Figure 14: An example of serial dilution sample preparation

3. For the 1st dilution, the wastewater sample are introduced into the 1st test tube and mix well tu ensure the aggregates are diluted completely. Micropipette is cleaned every time being used. 0.5ml of sample from the 1st dilution are then taken into the slides by applying the wet mount slides technique.

4. The prepared slides was put at the Stage Plate and the Abbe Condenser was adjusted to control the diameter of the beam of light entering the lens system. By changing the size of the iris and moving the lens toward or away from the stage, the diameter and focal point of the cone of light that goes through the specimen can be controlled. **Fig. 14** shows an example of prepared sample of serial dilution

5. The ViS ver .2.90 software was switched on and set into live video recording. The image of slides are appeared on the screen and the Objective Lens were adjusted at 10X magnification.

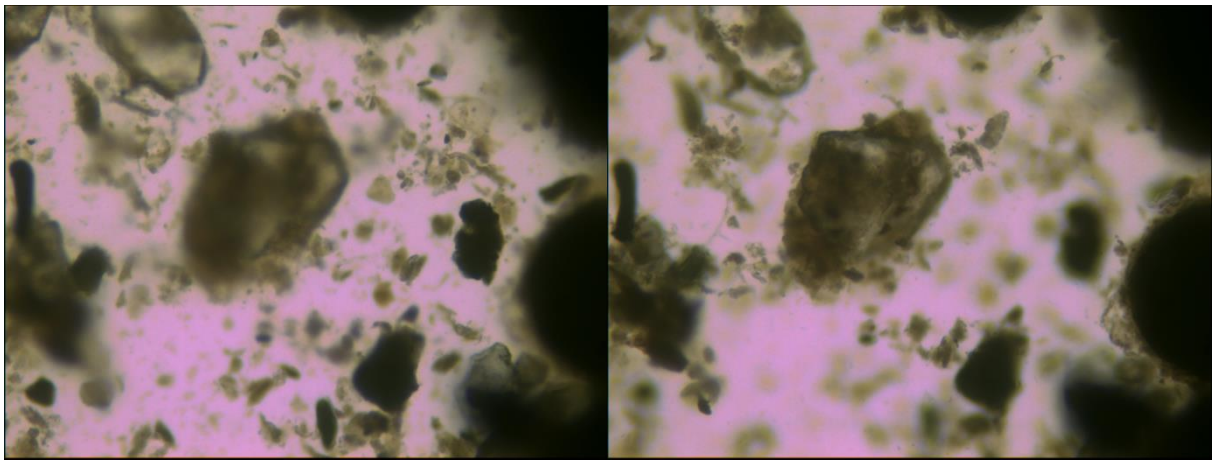


Fig. 15: Sample images of diluted sample (1ml distilled water) adjusted focus for designated image acquisition

6. The focus of the image are adjusted as shown in **Fig.15** with Diopter Adjustment to change the focus on one eyepiece to compensate for the difference in vision. The Fine Focus is the knob used to fine tune the focus on the specimen. It is also used to focus on various parts of the sample.

7. Images of sample are captured by using the "Capture Image" tools in the software. Each slides are divided into 4 section of image (top left and right, bottom left and right).

8. Images from each slides are saved in seperated folder for further analysis.

9. Steps 3 until 8 were repeated by serial dilution until the fourth sample.

10. The withdrawn sample image were analyse using ImageJ — Version 1.47 software and changed into 8-bit image for particle image analysis.

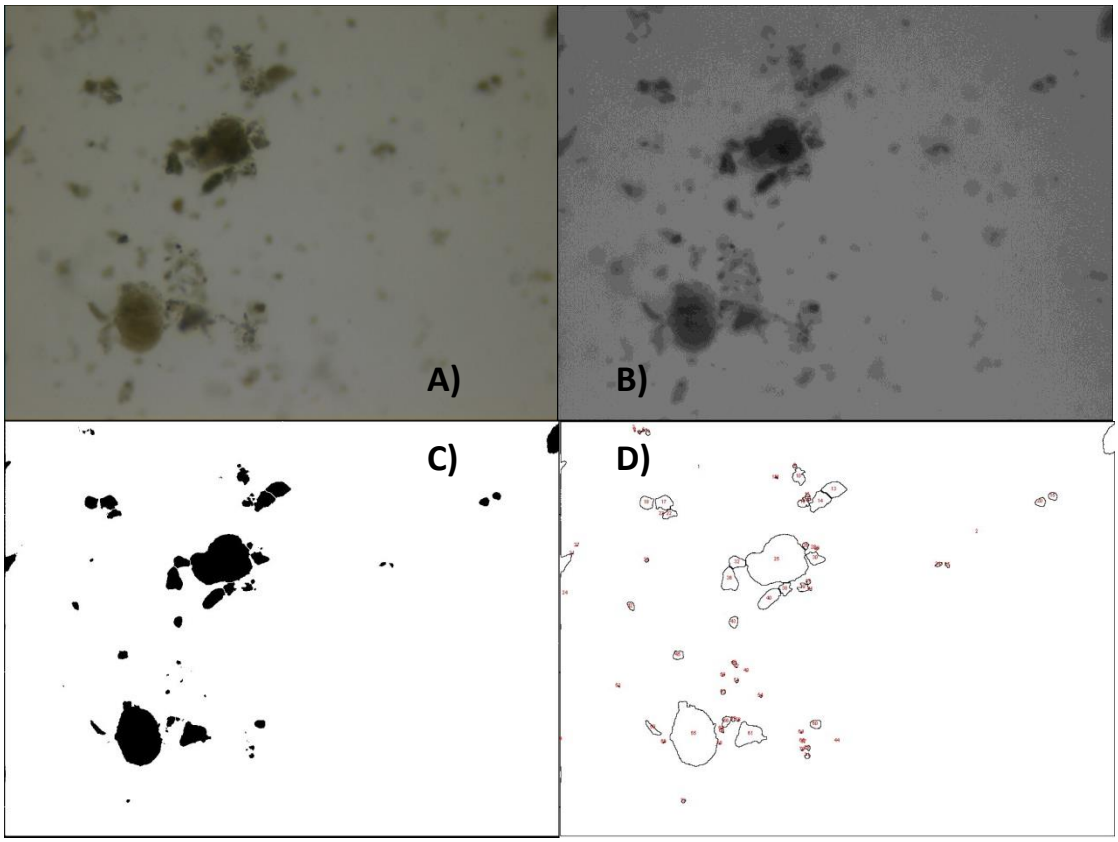


Fig. 16 Sample image analysed using original image (a) with 8-bit image (b), threshold (c), and cell counting (d)

11. The images were analysed using thresholding, segmentation and cell counting tools to identify the aggregates and filaments as shown in **Fig. 16**

12. Steps 3 to 11 were repeated using different amount of dilution and number of serial dilution to obtain the best sample image.

13. The data were recorded and analyzed for calculation and development of the project.

CHAPTER 4

RESULT AND DISCUSSION

4.1 EXPERIMENT RESULTS

4.1.1 First run

Variables: Dilution between distilled water with 1ml of POME sample (non-serial dilution)

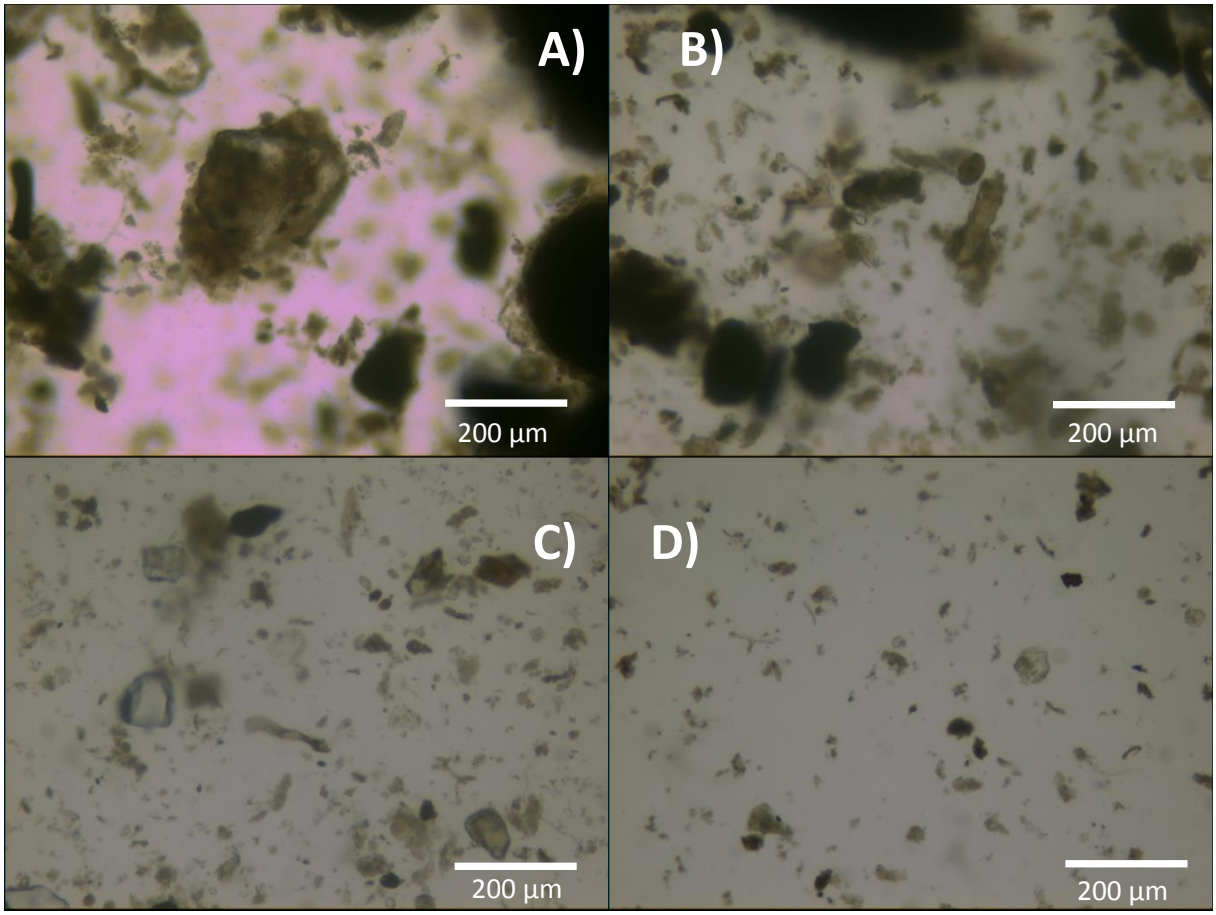


Figure 17: A) sample images of POME dilution with 1ml of distilled water B) 2ml of distilled water C) 3ml of distilled water D) 4ml of distilled water

4.1.2 Second Run

Variables: 1ml of distilled water with 1ml of POME sample (serial-dilution)

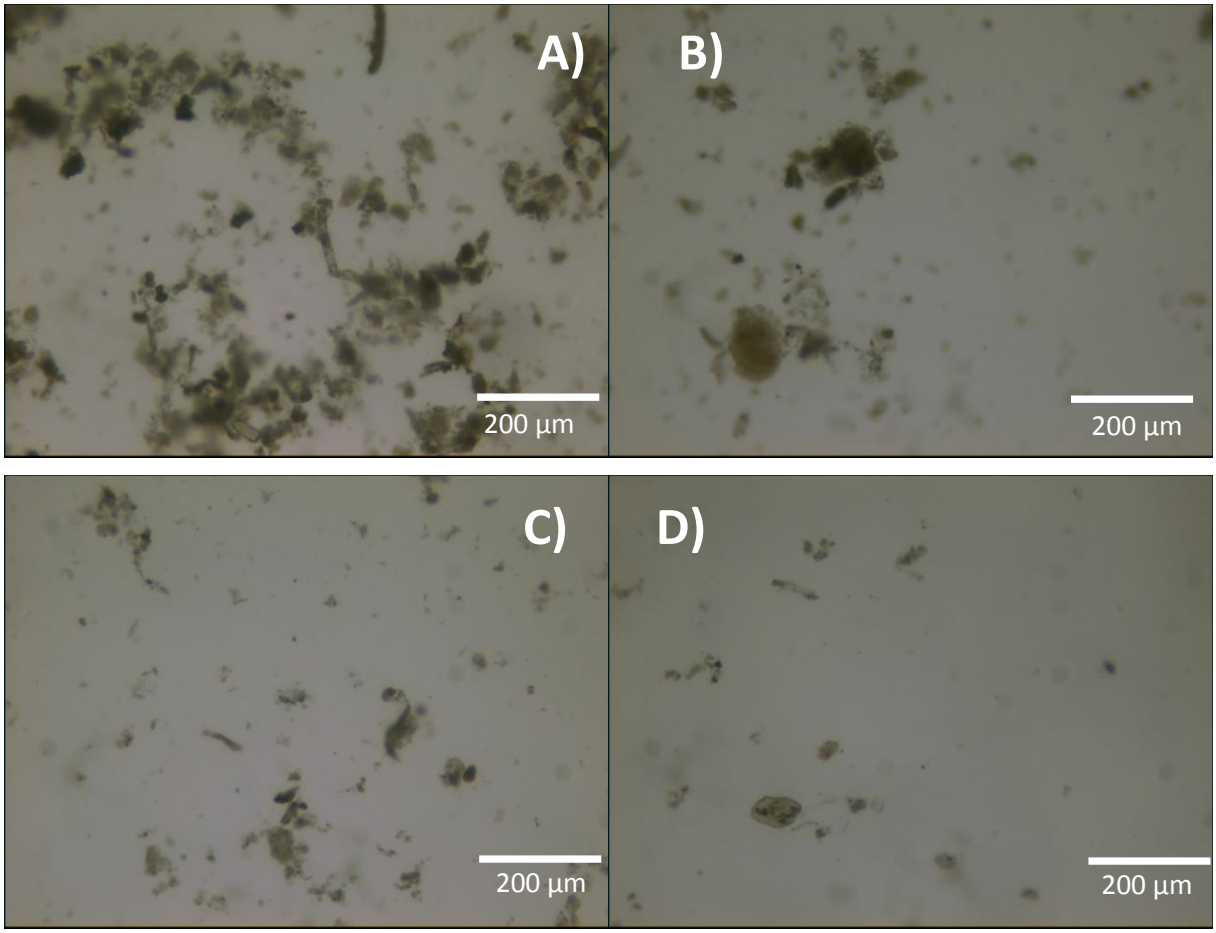


Figure 18: A) sample images diluted with 1ml of distilled water at first dilution B) second dilution C) third dilution D) fourth dilution

4.1.3 Thresholding and cell counting

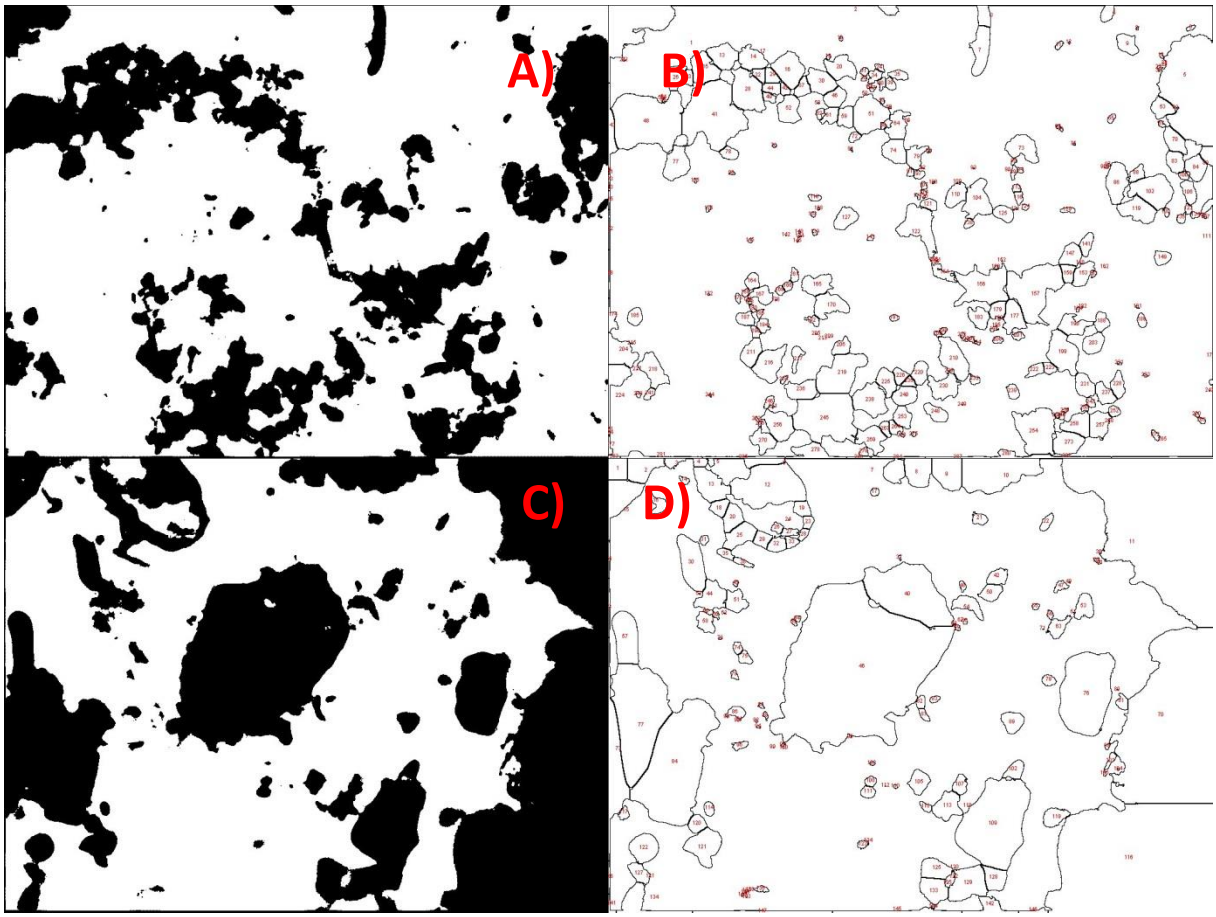
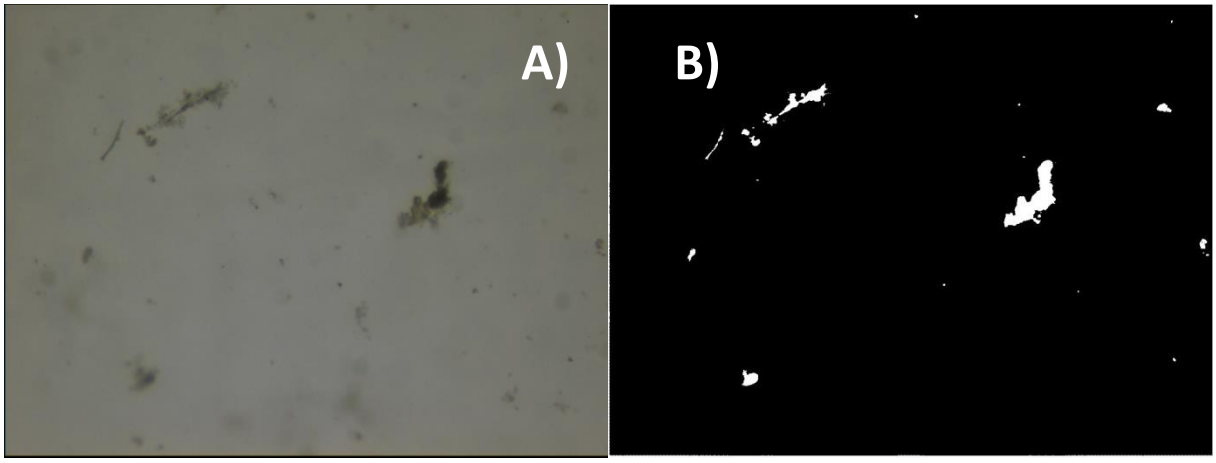


Figure 19: A) images from figure 16A with thresholding B) with cell counting C) images from figure 15A with thresholding D) with cell counting

4.1.4 Length-Width ratio



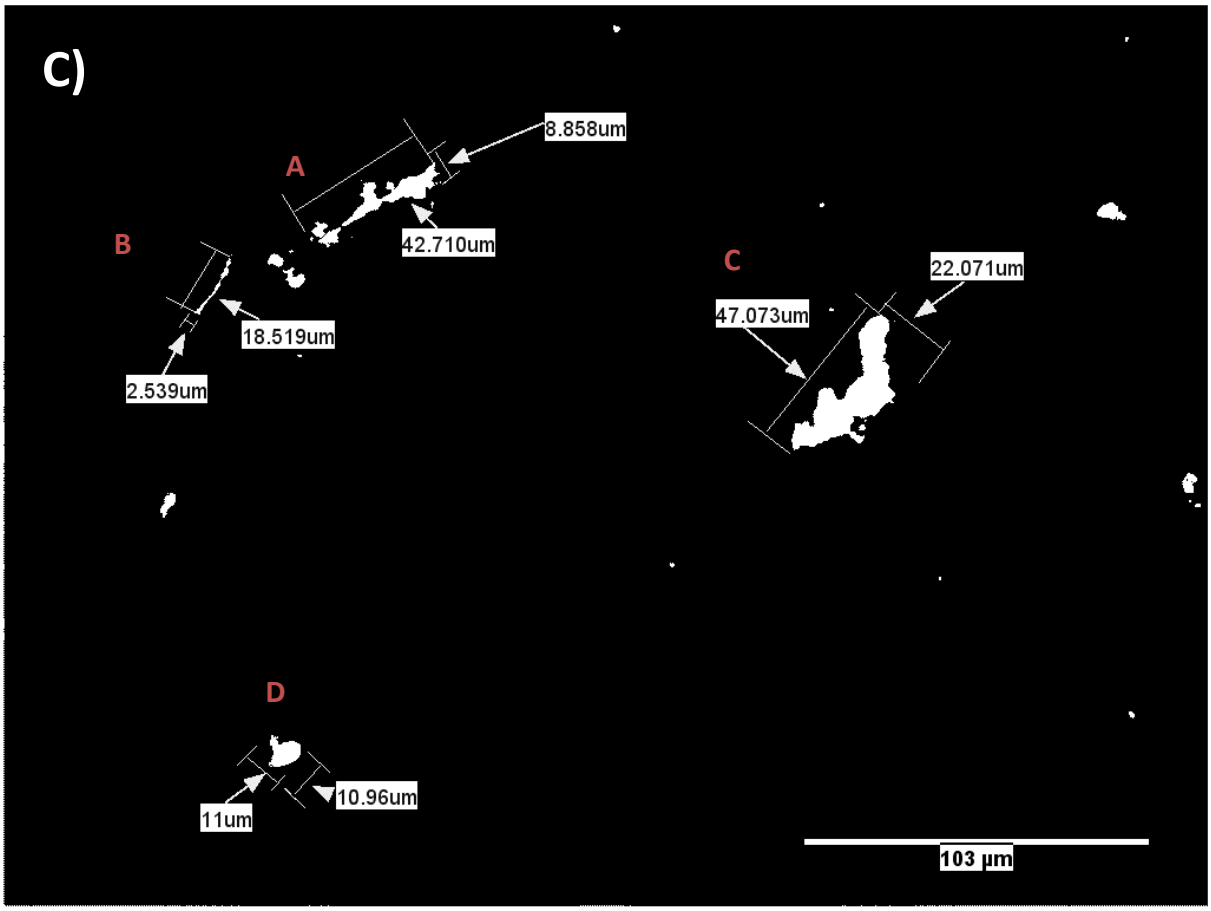


Figure 20: A) original sample image after dilution B) thresholding and subtract background C) measuring the aggregates length and width

Aggregates	Length	Width	L/W
A	42.710	8.858	4.822
B	18.519	2.539	7.294
C	47.073	22.071	2.133
D	10.96	11	0.996

Table 1: sample dataset of aggregates length and width ratio per image

*If L/W is greater than > 3.000 considered aggregates

*If L/W is smaller than > 3.000 considered filament

4.1.5 Average area and Perimeter

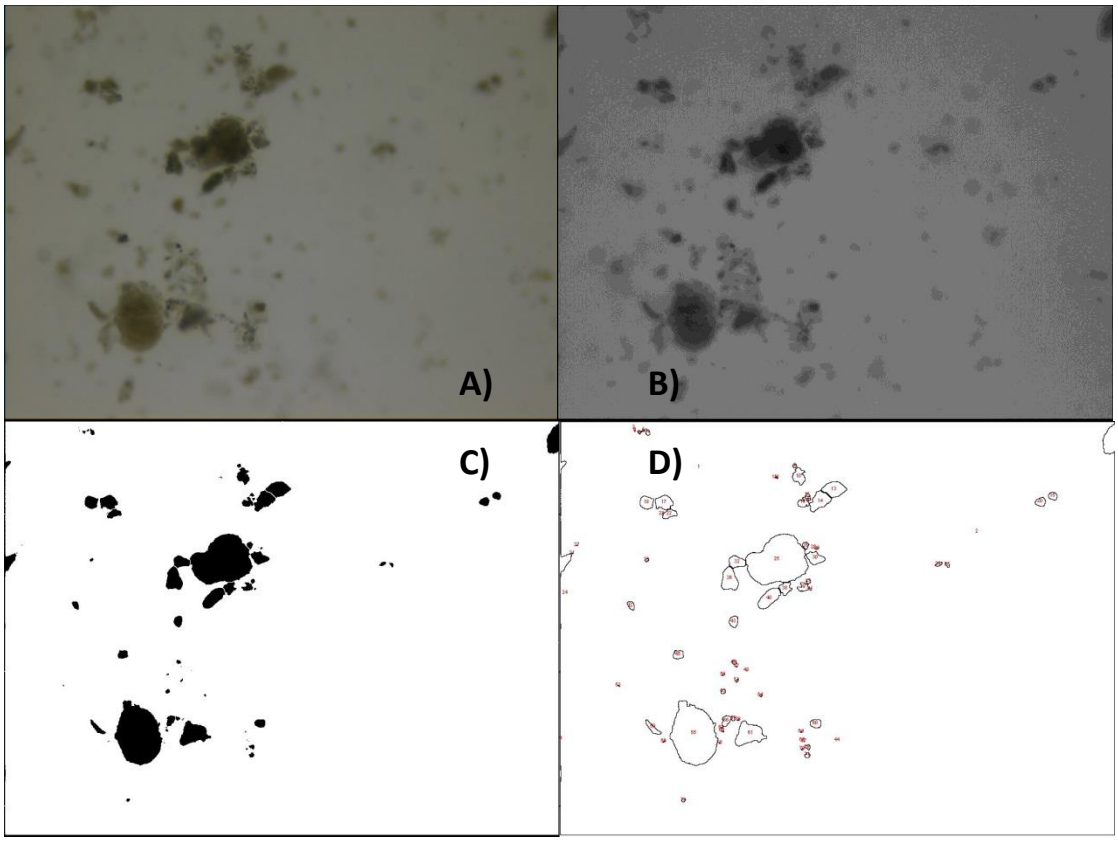


Fig. 21: Sample image analysed using original image (a) with 8-bit image (b), threshold (c), and cell counting (d)

From the images recorded above, the area and perimeter of each cell counted can be determined by "Measure" tools in the software. **Table 2** below are tabulated by using Fig. 19 (D) as reference.

Table 2: Fig. 19 (d) Size analysis

Cell number	Area (μm^2)	Perimeter (μm)
1	1035	1432.818
2	1831	1871.397
3	1	2.828
4	66	33.799
5	12	11.899
6	1	2.828
7	1	2.828
8	25	23.799
9	40	22.971
10	482	103.539
11	7	11.314
12	2	4.828
13	879	121.681
14	892	128.51
15	180	49.698
16	37	25.213
17	643	108.468
18	465	84.426
19	241	77.255
20	232	58.77
21	19	16.728
22	301	80.326
23	1	2.828
24	1033	653.463
25	7520	364.375
26	105	39.698
27	2	5.657
28	1	2.828
29	13	12.485
30	529	97.64
31	3	5.657
32	503	95.154
33	31	20.385
34	67	34.627
35	42	27.799
36	960	128.711
37	51	27.456
38	366	88.569
39	214	70.426

40	938	127.439
41	12	11.899
42	143	47.698
43	260	62.184
44	4557	2865.314
45	243	63.941
46	48	25.799
47	2	4.243
48	1	2.828
49	515	503.113
50	11	12.485
51	18	18.142
52	5	8.485
53	51	24.971
54	13	13.314
55	7304	378.517
56	233	64.184
57	45	25.556
58	7	11.314
59	264	85.64
60	256	60.284
61	1577	182.995
62	5	7.657
63	18	15.899
64	5	7.657
65	2	4.828
66	4	9.899
67	1	2.828
68	3	5.657
69	61	31.213
70	10	10.485
71	64	32.627
72	33	20.971

Total number of cell : 72

Total area: 2503.7 μm^2

Average area: 55.524μm^2

Average permieter: 16.061µm

From the data above, we could find the average size of aggregates by dividing total area per number of cell counted.

$$\frac{Total\ area}{Total\ number\ of\ cell} = \frac{2503.7}{72} = 34.774\mu m$$

Range size of aggregates: 10 - 40 µm

$$\frac{40\mu m - 34.774\mu m}{40\mu m} = 0.13065$$

$$0.13065 \times 100 = 13.065\%$$

Based on the calculation, we can assume that the size of POME aggregates in this sample is 13% less than the average size of aggregates in other wastewater system.

Table 3 below is generated from 32 sample images diluted with 1ml distilled water

No. of image sample	Total number of aggregates	Total area of aggregate (µm^2)	Average size of aggregate (µm^2)	Percentages of aggregate size (%)
1	341	19556.1	57.35	-43.37
2	231	14590.4	63.16	-57.90
3	287	19858.3	69.19	-72.98
4	118	2225.54	18.86	52.85
5	60	1632.27	27.20	31.99
6	151	1699.55	11.26	71.86
7	123	1512.23	12.29	69.26
8	217	2677.07	12.34	69.16
9	203	2582.71	12.72	68.19
10	122	2440.82	20.01	49.98
11	99	1579.42	15.95	60.12
12	187	2102.83	11.25	71.89
13	92	1772.63	19.27	51.83
14	133	1887.97	14.20	64.51
15	91	1881.18	20.67	48.32
16	116	1451.85	12.52	68.71
17	140	1482.74	10.59	73.52
18	43	1182.2	27.49	31.27
19	200	1658.96	8.29	79.26
20	117	3045.7	26.03	34.92
21	152	2808.1	18.47	53.81
22	145	3711.9	25.60	36.00

23	21	49.98	2.38	94.05
24	105	2705.1	25.76	35.59
25	122	5096.4	41.77	-4.43
26	160	3242.8	20.27	49.33
27	143	6693.8	46.81	-17.02
28	115	2981.6	25.93	35.18
29	87	3942.8	45.32	-13.30
30	5	29.193	5.84	85.40
31	59	438.34	7.43	81.43
32	191	4319.5	22.62	43.46
Average =	**136.75**	**3838.749**	**23.71**	**53.78%**

From all the experiment done within the time, 32 images are selected as the best image of aggregates and filament. These images are analysed based on *aggregates parameter and area*, followed by *total number of filaments/aggregates* exist per sample, *total area of filaments/aggregates* exist per sample, *the average size of filaments/aggregates* exist per sample, and *the increase/decrease percentages of filaments/aggregates* compared to its standard size in wastewater sytem. In a nutshell, the aggregates size in POME sample are much smaller (23.71 µm) and edges (+53.78%) than standard aggregates size. This shows that the microorganism in the activated sludge are in such poor environment resulting less efficient waste water treatment system.

4.2 DISCUSSION

4.2.1 Activated sludge monitoring through microscopic examination

The sludge settling ability is considered one of the main problems in especially in activated sludge (AS) systems and is commonly measured by the sludge volume index (SVI). Therefore, sludge flocculation, stability, aggregates size, morphology, and chemical composition are crucial to the efficient operation of an AS system. The complex nature of microbial communities, imbalances between the different types of bacteria may take place and disturb the plant with economical and environmental consequences. For this reason, aggregates analysis by microscopy inspection is a useful way for a fast malfunction diagnosis.

The success of a given AS system depends on a correct balance among floc-forming and filamentous bacteria. Shape, structure, and strength of AS flocs are three important characteristics regarding morphological characterization. Shape varies in its regularity,

roundness and compactness, among others as shown in **Figure 17** . If the flocs present highly irregular shapes, or present an open nature, consequently, the sludge settling velocity is reduced.

Bulking can cause severe operational problems, increasing the treatment costs and lowering the final effluent quality. The most critical problems involve poor settling ability, risk of sludge washout with the final effluent and deteriorated dewatering and thickening properties of the sludge. microbial aggregates characteristics, including their internal structure, chemical composition and microbial ecology, determine the transport properties and chemical reaction rates, and affect the overall performance of the treatment processes.

4.2.2 Aggregates-Floc characterization

It is known that the characterization of different types of bacteria is able to be performed using image microscopy coupled to analysis procedures. Measurement procedures are used in filamentous bacteria identification. The length-width ratio and roundness of particles in wastewater sample will differentiate the existence of a permeability barrier in bacteria based on the chemical and physical properties of the cell wall. Although a large variety of the filamentous microorganisms present in AS systems are permeable, a significant amount of negative bacteria is also commonly observed in AS systems. This characteristic is helpful to determine the filamentous bacteria contributing to poor settlement of AS flocs, by bulking or foaming.

4.2.3 Filament-Aggregates identification

The aggregated and filamentous biomass contents and morphology were assessed by thresholding and measuring its length and width on microbial microscopy. **Table 1** presents an example of dataset of length-width ratio used in this work. By measuring the size of the aggregates and filament, justification can be made on the system efficiency with the size of microbial organisms. The ratio also beneficial to determine the designated floc/aggregates which suits the system.

For example, if compact flocs are present, bacteria are stacked close to one another, leading to higher settling velocities. It should be noticed that the air supply in the aerated tank and the presence of pointy filamentous bacteria leads to flocs irregularly shaped. Typical floc characteristics include irregular shape, broad distribution of particle sizes, fragile and easily

compressible, large specific surface areas, inhomogeneous distribution of internal mass, networked structure, and poor dewaterability.

Overproduction floc-forming bacteria, and filamentous bacteria can lead to operational problems. These problems arise mostly when the operating conditions are not perfect mainly in terms of organic load, nutrients, and oxygen supply.

4.2.4 Diluting the sample

The 1st and 2nd dilution of experiment is meant to compare the effect of distilled water dilution in the image generated. In other word, it compares the right amount of serial dilution for more contrast and decreasing debris. From the cell counting images in the result section, the software will monitor the number of aggregates and filament in total per sample. The auto cell counter is useful to determine aggregates characteristics visually. It can be concluded that the image anlaysis through dilution technique are much better compared to the wastewater sample image alone. The dilution effect promotes lower concentration, hence better image acquisition in the wastewater sample.

4.2.5 Average area vs Length-Width Ratio

Computers are key equipments for the analysis of large amounts of data, for tasks requiring complex computation, and for the extraction of quantitative information, opposite to the qualitative evaluation of human analysis. Today, the automatic analysis of numerical images captured by digital cameras enables to extract quickly quantitative information. Thus, as a basic concept, image processing and analysis is the extraction of significant information from images, by means of digital image processing techniques.

The nature of computer in image analysis is to ease human and extract information quickly as well. Table 2 and Table 3 are generated automaticly by comparing the value side by side, thus the calculation on average-size of aggregates can be formed. However, the length-width ratio technique could be apply in such manner due to its manual measurement and low accuracy measurement. Instead of losing time for measuring the aggregates manually, the automatic average size aggregates are formed in Table 3.

Nevertheless, both technique shows a great discovery in image analysis method by proving its gravity in data extraction technique.

4.3 PROBLEM ENCOUNTERED

There are several problems encountered in conducting the experiment. These problems might affect the accuracy of experimental results.

4.3.1 Low resolution camera.

The biggest challenge in conducting the experiment is the equipment maintenance. The camera attached to the microscope has lower resolution compared to the objective lens. The focus on the camera are not sync with the focus on the eyepiece. The image of sample has to be refocused everytime looking through the eyepiece.The duration of readjust and refocus for evey image taken consume extra time for analysis. Hence, it is very difficult to maintain the sample exposure at the same time to analyse every sample in each run of experiment.

4.3.2 Period of Sample Collection

Various literatures mentioned that they can relate between aggregates behaviour against its time. However, the parameters such as the frequency of wastewater sample collected, the collection point, and the type of wastewater are varied in the examples of previous studies. Therefore, the aggregate results taken by previous study could not be compared due to limitations in the parameters. It is hope that the wastewater sample could be taken weekly or monthly, and the behaviour of aggregates inside the system could be monitored.

4.3.3 Microscopic magnification

Literature stated that the images taken from the lab microscopy with 400x total magnification (D. P. Mesquita, A. L. Amaraa, E. C. Ferreira, 201). However, due to limitation of equipment, the microscope can only be magnify to 40x magnification. It is observed that due to low resolution of attached digital camera, the image on the screen at 40x magnification are out of focus. Since there is no sufficient equipment to control this, the microscopic images are taken at only 10x magnification.

4.3.4 Accuracy of data and calculation

Throughout the analysing the information process, two major issues are ; the pixel-micrometer ratio, and calibration scale.

For every measurement using the ImageJ software are calibrated with 1 pixel/μm. However, from the eye of experts in microscopic analysis, this ratio will cause differences with the real

ratio that supposeedly been used in the software. This problems are discovered near the end of this project, and all the re-measuring and re-calculating may jeopardize the project's milestone and its completion schedule. Due to this matter, the Length-Width Ratio measurement as shown in Table 1 couldn't be used because of the error percentage are too high when parallax error are included when manually measure the length and the width.

The calibration scale are also missing in the images sample that been analysed afterward because of the same problem as well. The error in calibrating its measuring ratio cause the scale to show different value everytime the scale bar are shown. Nevertheless, the issue of calibration ratio are cause by systematic error and this error can be prevented when the exact pixel-ratio value are obtained.

The data measured can be more accurate when this issue are been solved. All the technique and method in this particular project are still valid and open a new window in microscopic tecnhique compared to other method in wastewater analysis, by proper reference and time management, this issues can be resolved.

CHAPTER 5

CONCLUSION

As a conclusion, this project is important as it deals with alternative ways of analyzing wastewater particle from industries. Image analysis is believed to be one of the effective ways to encounter the current problem with the conventional ways of treating the effluent by observing the aggregates and filamentous bacteria character . The imaging technique in this project are valuable to enhance the future activated sludge monitoring in the wastewater both economically and scientifically.

It can be said that this project is showing a good progress and expected results are obtained from the runs of experiments conducted. Although there are several problems encountered during the experiment, it will not cause too much error in the experiment. The experiments will be resumed and it is hoped that this project will come to a positive conclusion.

The project is within capability of a final year student to be executed with the help and guidance from the supervisor and the coordinator. The time frame is also feasible and the project can be completed within the time allocated. It is hoped that the acquiring of equipment and materials needed for the experiment runs smoothly for the accomplishment of this project at the end.

This project showed that microscopic analysis is considered a powerful technology with great potential of application in wastewater treatment especially in AS systems. Over the years, the number of image analysis studies for biomass and sludge characterization is ever increasing, aiming at clean and safe final effluents in wastewater treatment, through the combination of operating parameters and image analysis information.

3.3 References:

[1] A.L. Amaral, E.C. Ferreira, "Activated sludge characterization through microscopy - A review on quantitative image analysis and chemo metric techniques," (2005) 247.

[2] D. P. Mesquita, A. L. Amaraa, E. C. Ferreira, "Activated sludge monitoring of a wastewater treatment plant using image analysis and partial least squares regression," (2013) 15-16.

[3] M. Verawaty, S. Tait, M. Pijuan, Z. Yuan, P. L. Bond, "Breakage and growth towards a stable aerobic granule size during the treatment of wastewater," (2013) 5338.

[4] D.P. Mesquita, A.L. Amaral, E.C. Ferreira, "Characterization of activated sludge abnormalities by image analysis and chemometric techniques," (2011) 236.

[5] Y.P. Ginoris, A.L. Amaral, A. Nicolau, M.A.Z. Coelho, E.C. Ferreira, "Development of an image analysis procedure for identifying protozoa and metazoa typical of activated sludge system," (2007) 2582.

[6] L. Smoczyn´ski, H. Ratnaweera, M. Kosobucka, M. Smoczyn´ski, "Image analysis of sludge aggregates," (2013) 413.

[7] (2007) Retrieved from City of Boulder/USGS Water Quality Monitoring: http://bcn.boulder.co.us/basin/data/BACT/info/TSS.html

[8] G. Bitton, "Encyclopedia of Environmental Microbiology", (2002) vol. 4. Willey International Publication, pp. 2605–2612.

[9] P. Madoni, "The Handbook of Water and Wastewater Microbiology", (2003) Academic press (Elsevier), 22, pp. 361–371